Croonian Lecture :—*On Globulins.*

By W. B. Hardy, M.A., F.R.S.

(Being the Croonian Lecture delivered May 25, 1905.—MS. Received April 9, 1907.)

Globulins are a class of proteids which occur in both animal and vegetable tissues. They are peculiar in the complexity of their relations to electrolytes. Insoluble in water, they are soluble in low concentrations of acids, alkalies, or neutral salts. In presence of acids the globulin is electro-positive, in presence of alkalies it is electro-negative, in presence of neutral salts it is electrically neutral. Electrically active globulin (*i.e.*, dissolved by acids or alkalies) is precipitated by minute amounts of neutral salts; also, no matter what its electrical state may be, or how dissolved, globulins are precipitated by neutral salts near the saturation point of the latter. The problem I propose to consider is their diversified relation to electrolytes.

Connected with this problem is another, namely, the relation of solutions of globulins to colloidal solution. Do they form hydrosols at all, and, if so, to what extent? Krafft urged the colloidal nature of soap solutions, because, within certain limits of temperature and concentration, they gelatinise, and have the vapour pressure of pure water. Kahlenberg and Schreiner, however, regard soap solutions as being crystalloid in character, because over the whole range of concentration the soap is a good electrolyte—it ionises and undergoes hydrolytic splitting, like other salts of a weak acid and strong base. Smits, again, by measurement of the vapour pressure over a wide range of concentration, is convinced that above a critical concentration the soap passes wholly into the colloidal state.

Exactly the same points arise in connection with proteid. Waymouth Reid has shown that the proteids in a solution exert no measurable influence upon the vapour pressure. On the other hand, solutions of globulins are relatively good conductors over the whole range of concentration through which it is possible to follow them. The dilution curve shows no break indicating a general change of state, even when concentration is pushed to the point where fluidity almost vanishes.

Again, colloids, as a class, are chemically inert. But globulins react actively with acids or alkalies to neutralise them. Globulin solutions, even at extreme concentration, form syrups and not true gels, except, perhaps, under special circumstances. Clearly, therefore, it is pertinent to ask whether globulins form colloidal solutions.

The globulin used throughout this research is that which is precipitated from ox serum by dilution and slight acidification.

Solution by Acids or Alkalies. Electro-positive or electro-negative globulin. When acid or alkali is gradually added to a suspension of globulin in water, the opaque suspension gradually changes to a clear transparent fluid. Between the opaque suspension, from which the globulin settles on standing, to the stage of limpid transparency there is no break. The most minute addition of acid or alkali (in the absence of salts) converts the suspension into a non-settling "solution" of low grade, which is opaque white from the large size of the particles of globulin dispersed throughout it. Further addition of acid or alkali raises the grade of solution. The globulin particles become smaller and smaller until complete transparency is reached.

The process can be reversed by dialysis, save that, at the lowest grade attainable, no precipitate settles in the absence of disturbing factors.

With the first addition of acid or alkali, and at the last stage of dialysis, the globulin is electrically active, so that it moves in an electric field, the direction of movement being that which one would expect if it combined with the acid or the alkali to form a salt, and the specific velocity is quite, or nearly, at its maximum value. A rise in the grade of solution is, on the whole, associated with a fall in the specific velocity, never in my experience with a rise in this value. The electric charge on the surface of the globulin particles therefore appears to reach maximal density when the solutions are still of exceedingly low grade.

These facts can best be explained by assuming that true salt formation occurs. This agrees with the view of all previous workers on the interaction of proteids with acids or alkalies (Sjöqvist, Bugarsky and Liebermann, and Cohnheim). Since globulins combine either with acids or bases, they have both an acid and a basic function—they are amphoteric electrolytes.

The globulin salts ionise in solution; therefore, in an electric field the entire mass of proteid moves. They also hydrolyse, but the hydrolysis offers special features resembling those which Jordis has pointed out in the case of sodium silicate, and Chevreul in the case of soaps. In both of these cases hyper-acid salts are formed, while hyper-acid salts are formed on dialysis of a solution of globulin by alkali, and hyper-basic salts on dialysis of a solution of globulin by acid.

By dialysis the degree of hyper-acidity or hyper-basicity can be raised, but, as Jordis finds in the case of silica, with continuously increasing difficulty. With the rise in the degree of hyper-acidity or hyper-basicity, the "grade" of solution diminishes, but, in the case of globulins, precipitation

does not occur. Electrically active solutions of globulin cannot be precipitated by dialysis, nor at any stage do they cease to be electrically active.

If G be used to denote globulin, the equation of hydrolysis or dialysis would be:

$$x\,\mathrm{GHS} + y\,\mathrm{HOH} = (\mathrm{GHOH})_y(\mathrm{GHS})_{x-y} + y\,\mathrm{HS},$$

or $$x\,\mathrm{GB} + y\,\mathrm{HOH} = (\mathrm{GH})_y(\mathrm{GB})_{x-y} + y\mathrm{BOH}.$$

In dialysis, the ratio x/y varies continuously. It is as indeterminate as the ratio between the combining salts has been found to be by Bödlander, Abegg, Sherrill, and others in the double salts of mercury and silver.

Clearly a relation of this kind agrees with van Bemmelen's definition of absorption compounds as chemical combination with variable composition.

Removal of water from a solution of acid or alkali globulin does not produce precipitation, and the dried gummy residue reabsorbs water and passes slowly again into a state of solution. Acid and alkali globulin, therefore, form, with water, solutions which have the feature characteristic of colloidal solutions, in that there are no saturation points.

In order to compare the solvent power of different acids or alkalies, it is necessary to fix upon some arbitrary point, such as the point of minimal opalescence. By the use of a system of controls and proper illumination this point furnishes very concordant values.

Measured in this way, it appears that for strong and medium acids solvent power is measured by the number of gramme molecules present, not by the number of gramme equivalents:

$$\mathrm{HCl} = \mathrm{H_2SO_4} = \mathrm{H_3PO_4}.$$

Very weak acids have a lower solvent power: $\mathrm{HCl} = 5\mathrm{H\bar{A}} = \pm 30{,}000\mathrm{H_3BoO_3}$.

These relations are explained by the very weak basic function of globulin. Salts with weak acids are much hydrolysed, and to reach the same grade of solution an excess of acid is needed in order to lower the degree of hydrolysis.

With alkalies, the weak alkali $\mathrm{NH_4OH}$ dissolves as well as the strong alkalies, owing to the fact that globulin acts as an acid of considerable strength.

The acid and basic functions were measured by the well-known methods—the catalysis of cane sugar and of methyl acetate—and the acid function found to be much the greater.

The molecular relation noticed above recalls the salts of amido acids:

$$\mathrm{R}\begin{smallmatrix}\mathrm{NH_2}\\ \mathrm{COOH}\end{smallmatrix} + \mathrm{HCl} = \mathrm{R}\begin{smallmatrix}\mathrm{NH_2HCl}\\ \mathrm{COOH}\end{smallmatrix}; \qquad \mathrm{R}\begin{smallmatrix}\mathrm{NH_2}\\ \mathrm{COOH}\end{smallmatrix} + \mathrm{H_2SO_4} = \mathrm{R}<\begin{smallmatrix}\mathrm{NH_2H_2SO_4}\\ \mathrm{COOH}\end{smallmatrix}.$$

2 H 2

Data derived from measurements of electric conductivity, and from the behaviour with indicators, support the view that true salts are formed, and that the globulin acts much more strongly as an acid than as a base.

Direct Measurement of the Specific Velocity of Globulin "Ions."—This was carried out by the boundary method.

As the basic function of a globulin is weaker than its acid function, the salts GHS (globulin + acid) will be hydrolysed more than the salts GB (globulin + base). Therefore, in the equation of hydrolysis given above, y/x will be larger for the former than the latter. Similarly, comparing salts with $H\bar{A}$ and with HCl, y/x will be greater for the former.

Now with increase in the value of y/x the size of the proteid particles increases also. By dialysis, the size can be increased until the particles diffract white light. The growth in the case of solutions of sodium silicate can be traced by the appearance of molecular states incapable of passing through a parchment membrane. In a similar way, by continuous addition of AgCl to a solution of NaCl, molecular states are produced large enough to diffract white light.

In each of these cases these large molecules are ionic, that is to say, they take part in the electric transport. And their specific velocity is exceptionally high; that of $(AgCl)_x\ Cl_y$ being 57×10^{-5} at 18°, whereas Ag at infinite dilution is only 58.

"Ions" of this order of magnitude have the properties of matter in mass. Each is defined by a surface and moves under the influence of a surface contact difference of potential. With their appearance the fluid ceases to be homogeneous. It has internal surfaces. I propose to call such ions colloidal ions, or pseudoions. Their specific velocity is high, and, within wide limits, is independent of their size, and is controlled by the laws of electrical endosmose.

These conclusions are borne out by the boundary measurements and by the electrical conductivity of colloidal solutions.

For instance: theoretically the proportion of pseudoions in the following solutions of globulin should be:—

$$\text{In } H\bar{A} > \text{in HCl}, > \text{in NaOH},$$

and the specific velocities at 18° were found to be 23×10^{-5}, $11{\cdot}5 \times 10^{-5}$, $7{\cdot}6 \times 10^{-5}$. By Ostwald's law of the relation of specific ionic velocity to the number of atoms in an ion, the value 23×10^{-5} is an utterly impossible one for an ordinary ion of the magnitude of the proteid molecule.

Solution by Neutral Salts.—Globulins are dissolved by neutral salts owing to the formation of molecular compounds (G.BS). These compounds are readily decomposed by water with liberation of insoluble globulin

(G.BS + HOH = G.HOH + BS). Therefore they are stable only in presence of a large excess of salt. Hence the solvent power of salts is from 200 to 500 times less than that of acids or alkalies. Hence, also, the presence of the globulin lowers the electric conductivity of the salt to only a small extent (2 to 6 per cent.).

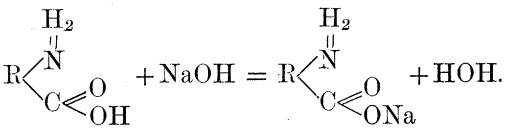
A double salt of the form AB.CD. ionises according to AB.CD $\rightleftarrows$ AB.C + D.

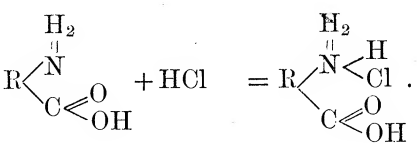
I have never succeeded in detecting any trace of such ionisation in the case of globulin and neutral salts; possibly owing to the extreme instability of the ions. The proteid does not move in an electric field, and it seems to take no part in the electric transport.

Owing to the insolubility of the dissociation products, the globulin can be precipitated from its solution with neutral salts by simple dilution. No degree of dilution will precipitate globulin from solution in dilute acid or alkali.

The compounds of globulin and alkalies (GB) are more readily dissolved by neutral salts than is simple globulin. Compounds of globulins and acids are insoluble by neutral salts, being decomposed with liberation of the acid.

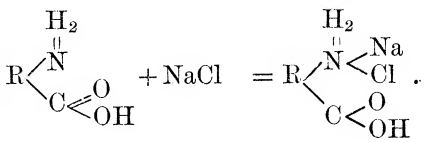
If on the analogy of an amido acid a globulin combines owing to the presence of the NH_2 and the COOH group, then it forms salts with alkalies by replacement of hydrogen, and with acids by the change of the trivalent nitrogen of the amido group to the pentavalent form.

i. 

ii. 

Where, then, does the neutral salt link on?

There are two possible places—the unsatisfied valencies of the nitrogen, or the unsatisfied valencies of the upper O of the COOH group. According to the oxonium theory, O has a maximum valency O$\equiv$, but O$\equiv$ is much less stable than N$\equiv$; therefore, one would expect the linkage to be by the amide group—

iii. 

And this would account for the fact that the acid can be turned out of the combination with globulin by an excess of neutral salt, but the alkali cannot be.

It is easy to prove by the boundary method that the globulin, when dissolved by salts, takes no part in the electric transport. Dissociation of the salt-globulin compound by the water may be regarded as being practically completely suppressed by the excess salt present. This being the case, the diminution of the electric conductivity of the salt by the globulin may be used as a measure of the fraction of the salt actually combined with it.

In the following table a few values are given. Comparison is made between solutions containing the same amount of salt per litre; but in the one case the solution is saturated with globulin, in the other case it is a simple solution of the salt in water. No correction is made for the diminution of the molecular conductivity of the salt owing to replacement of a portion of the water by globulin, since it would amount to less than 0·01 per cent. No. III is of the nature of a control, to prove that electrolytic impurities adherent to the purified globulin may be neglected. The salt solution was made up with a dialysate in equilibrium with the suspension of globulin.

Serum centrifuged, diluted to 10 vols., globulin precipitated by HĀ. The precipitate suspended in a large volume of distilled water and collected by the centrifuge, and re-suspended in water freed from gases by boiling.

Specific conductivity of the water employed, 6×10^{-6} rec. ohm, 18°.

I. Ox globulin suspended in water. 100 c.c. = 4·08 grammes dry globulin. Temperature, 18° :—

Salt.	Normality.	Specific conductivity, salt globulin, $\times 10^5$.	Specific conductivity, salt water, $\times 10^5$.	$\frac{\text{K salt globulin}}{\text{K salt water}}$.
K_2SO_4	0·1716	1445	1539	0·938
„	0·1679	1411	1506	0·937
NaCl	0·27	1850	1950	0·949

Specific resistance of the globulin suspension $3{\cdot}1 \times 10^{-5}$.

II. Sheep globulin. 100 c.c. = 4·4 grammes :—

NaCl	0·2659	2145	2280	0·938
„		2150	2278	0·943

The measurements in II were duplicated in order to test the degree of accuracy of the measurements of volume.

III. Ox globulin dialysed until in equilibrium with water saturated with toluol :—

$MgSO_4$	0·11	517	543*	0·95

In this case the salt solution (*) was made up with the dialysate, not with water.

[*Note.*—In a recent paper* it is stated that globulin in solution does not alter the

* Mellanby, 'Journ. of Physiology,' vol. 33, p. 354, 1905.

conductivity of the salt used to dissolve it. The conductivity of the salt "is the same as that of a similar solution in pure water." This statement is directly contrary to my own observations. It leads me to add a few words as to the precautions which are necessary in dealing with proteid solutions.

Electrodes coated in the ordinary way with platinum black are quite useless. The proteid in their immediate neighbourhood is changed very rapidly and very drastically. An instance selected at random will show the magnitude of the effect.

18°. Globulin dissolved by dilute NaOH. 3·3 per cent. globulin. Readings at intervals of two minutes approximately :—

2560 ohms.
2542 „
2537 „
2530 „
2522 „
2538 „

The rise in the last observation followed upon slight shaking of the cell to wash the changed portion of the solution away from the electrodes. Platinum grey electrodes, prepared as Whetham directs, give no trouble. Readings with globulin solutions of all kinds remain quite constant after being for 48 hours and longer in contact with them.

Even with platinum grey electrodes, however, it is necessary so to adjust the cell that the observed resistance does not fall under about 500 ohms. With a lower resistance, and therefore a larger current, the readings become unsteady and too low.]

On the assumption that the whole of the drop in electric conductivity is due to association of the salt with the globulin, the above figures show that, at the particular concentration and temperature, 1 gramme of dry globulin combines with 33×10^{-5} gramme equivalent of NaCl and 26×10^{-5} gramme equivalent of K_2SO_4, while to dissolve 1 gramme of dry globulin 10×10^{-5} equivalent of alkali, or 18 to 36 equivalents of acid are needed. The figures for salt and acid are of the same order of magnitude, and this is what might be expected if salt and acid combine in the same way with the proteid.

Appendix.

(*Added December*, 1906.)

Viscosity of Solutions of Globulins.—The measurements of this value, very briefly touched upon in the lecture, have been amplified. They show the following interesting features :—

In dilute solutions of globulins the viscosity, as measured by the rate of flow through a capillary tube, is of the same order of magnitude for each of the three types—acid, alkali, and salt globulin.

With increasing concentration the viscosity increases, but the increase is much greater for alkaline globulin than for acid globulin, and for acid globulin than for salt globulin. The difference is very striking; thus, at a concentra-

tion of 7·59 grammes globulin per litre, the transpiration times are in the ratio—

Water	1
$MgSO_4$ globulin.....	4·66
HCl „	15·5
NaOH „	67·9

In solutions of salt globulin, the globulin particles do not carry electricity; "ionic" proteid is completely absent, therefore the higher viscosity of acid and alkaline globulin solutions is connected with the presence of "ionic" proteid, that is, of very large molecules carrying a charge.

Since globulin acts much more strongly as an acid than as a base, there will be in similar solutions of HCl globulin and NaOH globulin a greater concentration of "ionic" proteid in the latter. Here, again, increased concentration of "ionic" proteid goes with increased viscosity.

These conclusions agree with the observations of Reyher* on the viscosity of solutions of soap, and of Sackür† on solutions of the proteid caseinogen. It would, however, be rash to fix upon the simple proteid or soap ion as the agent. The effect of dilution, and of varying proportions of acid or alkali, make it possible that the high viscosity is due to the presence of the molecular complexes, which carry a surface charge, and which I have called "colloidal" ions, or pseudoions. Thus, further addition of small quantities of ammonia, beyond the amount necessary to dissolve the globulin to a transparent solution, *decreases* the viscosity. Ammonia is a very weak base, which, in solution, has a specific molecular conductivity much less than that of its compound with globulin. One cannot, therefore, refer the diminished viscosity to diminished ionisation of the ammonia-globulin compound, but the optical properties of the solution show that slight excess of ammonia does diminish the size of the particles of the globulin. It raises the "grade" of the solution.

A comparison of the different types of solution of globulin raises, in a special way, the question of the meaning to be attached to the phrase "colloidal solution." Mixtures of very diverse character have been classed as colloidal, some because they form jellies, others because they are physically heterogeneous, others because the osmotic pressure is exceedingly low, others, again, because the electric conductivity is abnormally low. Of these, perhaps, the only constant attribute is physical heterogeneity. Solutions which present any of the other distinctive features of the colloidal state always contain particles which are large enough to scatter light.

* 'Zeits. f. Physik. Chem.,' vol. 2, p. 743, 1888.

† *Ibid.*, vol. 41, p. 672, 1903.

Amongst the attributes of colloidal solutions is the possession of an abnormally high viscosity. Judged in this respect, the salt globulin solutions are only slightly colloidal as compared with the solutions of alkali or acid globulin. They differ also from acid and alkali globulin in the relatively greater definiteness of the relations between the components. Dilution of a solution of salt globulin, for instance, brings about a sharp separation of solid and liquid. No such clean saturation points are found with the other two systems. The definiteness of the relations makes it possible to compare the system—neutral salt, water, globulin with other similar three-component systems.

Data sufficient for the purpose can be obtained from the measurement of the solubility of globulins in aqueous salt solutions made by Osborne and Harris,* and by Mellanby.† The curves which these authors give fail to show the true relations, since only two co-ordinates are used, namely for the salt and the globulin; the third component, water, is neglected. For the following curves, trilinear co-ordinates are used in the manner recommended by Stokes.

Fig. 1 shows curves plotted to scale from values derived from Osborne and

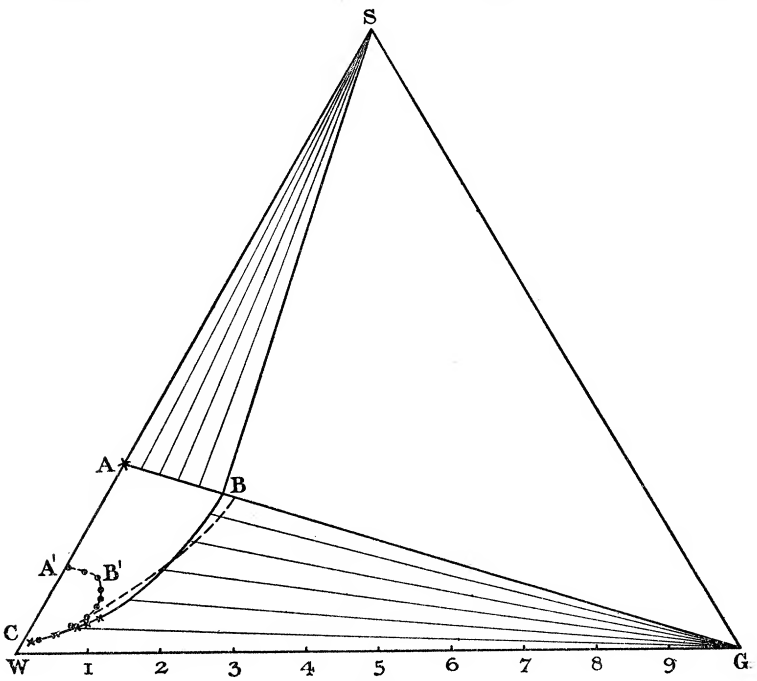


Fig. 1.

* 'American Journal of Physiology,' vol. 14, 1905.

† *Loc. cit.*

Harris.* S is pure salt, W pure water, G pure globulin (edestin). The points on the curves, fixed by actual experiment, are shown in the usual way by dots or crosses. A′B′C is the curve for Na_2SO_4, ABC the curve for NaCl. The broken line extending to just beyond B is the probable form of the curve for H_2SO_4. The diagram is drawn as an isotherm for NaCl, water, edestin, the areas for the other salts being left out. The context is not very clear on the point, but the temperature of observation seems to have been 20°.

Each curve clearly consists of two distinct parts, AB, which is in equilibrium with solid salt, and BC in equilibrium with globulin crystals. The whole surface, therefore, consists of certain areas:—ABC, all points within which represent a homogeneous state, namely, a fluid solution of globulin, salt, and water; ASB, which is an area of heterogeneous states. Each point in it represents a mixture which cannot exist as a uniform state, but divides into two phases, one a fluid solution on the curve AB, the other a solid which is pure salt. WBG, again, is an area of mixture which cannot form a uniform state, but which divides into a fluid solution on CB, and into globulin crystals. SBG, each point within which represents a mixture which divides into a fluid having the composition of B and solid salt and solid globulin.

In fig. 2, portions of three isotherms for NaCl, water, serum globulin are

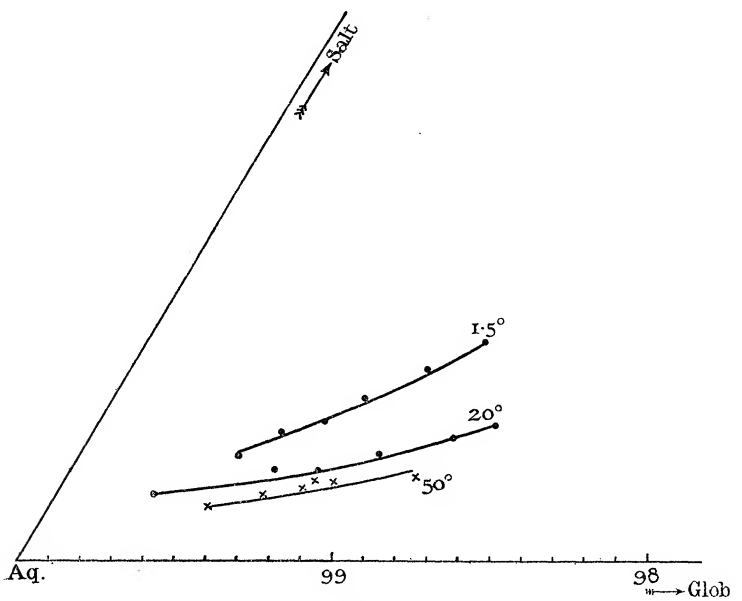


FIG. 2.

* *Loc. cit.*

plotted to scale, the data being derived from Mellanby's paper. They cover only a very small fraction of the whole curve, ABC, but they are sufficient to show, (1) that the curve encloses an area which is placed on the line WS, as in the preceding case. This follows from the form of the curve, and from the fact that if sufficient salt be added, a point is reached on the line WS where salt solution is in equilibrium with solid globulin. (2) That the area enclosed increases with a rise of temperature.

This last fact, together with some observations of my own, enable us to approximate roughly to an isotherm for edestin at, say, 30°. When edestin is present in sufficient quantity, on warming a further amount passes into solution, so that we have a series of solutions in equilibrium with crystals. Beyond a certain temperature the crystals fuse, and we then have two fluid layers in equilibrium, the lower layer (as I have found by actual analysis) containing all three components.

Fig. 3 is a diagram of an isotherm at a higher temperature; it shows the area ABC enlarged, and it shows a new area, ROP, due to the appearance of

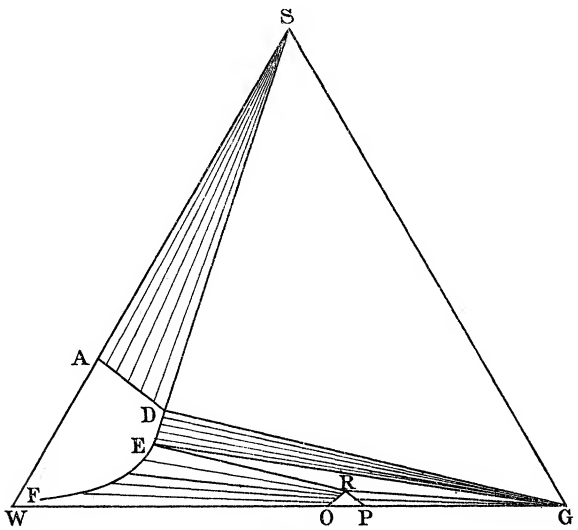


Fig. 3.

a second series of solutions (the melted crystals). Of the shape of this area we know nothing. The curve ABC now contains three parts, AD in equilibrium with S, DE with G, and EF with the solutions which lie on OR.

Fig. 1 does not represent the conditions for blood globulin at any temperature, since, in presence of water alone, a solid solution of water in globulin is formed. The tie lines from the curve BC, therefore, instead of focussing at G, end on a curve of unknown slope, which starts from about

the middle of the line WG. In other words, the isotherms of blood globulins, over the region where we have any information to guide us, are of the form shown in fig. 4.

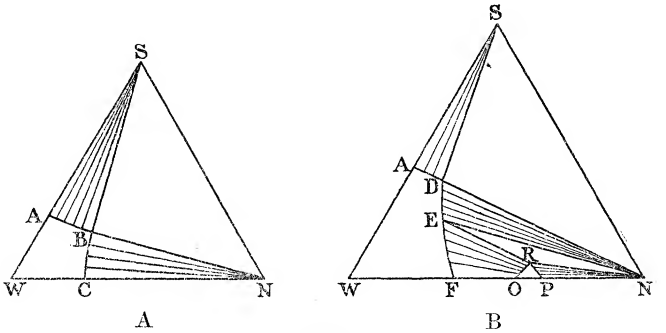


Fig. 4.

This is as far as the known facts carry us. It now remains to compare the system with a similar one. Such a one is found in water, sodium chloride, and succinic nitrile, which has been studied by Schreinemakers.* In both there is a pair of immiscible bodies, salt, and globulin, or salt and succinic nitrile, each of which is partially miscible with the third substance, water. Fig. 4, A and B are two isotherms reproduced from Schreinemakers' paper. It is obvious that, in all essential features, they resemble the isotherms for globulin-salt-water already given.

It is clear, from this comparison and from the low viscosity, that globulin-salt-water is a system showing few abnormal features. It manifests decisive points of equilibrium, which resemble those in a comparable and purely crystalloid system. The other two globulin systems are as decisively abnormal, especially in the high viscosity, and the absence of definite transition points, and it is significant that, regarded from the purely physical standpoint, the essential difference should be the presence in the latter of large molecular aggregates, each carrying an electric double layer.

The presence of internal electrified surfaces adequately accounts for the high degree of inertia which obscures the transition points. What is the source of the electrification? In all cases in which it can be traced, the charge is due to molecular interaction of the type classed as chemical, and of that particular chemical type associated with the decomposition of neutral electricity, to which the name "ionisation" has been given. Burton† clearly shows that metals form hydrosols because they react with the solvent to form hydrides or hydroxides. Acid globulin, alkaline globulin, and soaps are salts.

* 'Zeits. f. Physik. Chem.,' vol. 23, p. 417, 1897.

† 'Phil. Mag.,' April, 1906.

which form colloidal solutions because one of the radicles is of large size, is almost or quite immiscible with the solvent, and therefore readily forms molecular complexes which have the electric sign proper to the radicle when dissociated from its fellow in the salt molecule.

Each colloid particle undoubtedly carries on its surface a charge, since, without exception, they move in a uniform electric field. But in the system formed by each particle there cannot be any free electricity, any resultant charge. If there were, the system would, by Earnshaw's theorem, be unstable, and settling would occur the more rapidly the greater the charge. Experiment shows the opposite to be the case. The charge on each particle, therefore, must be bound by an equal and opposite charge on the liquid face opposed to it. The condition of double electric layers is therefore realised.

It has been shown by Waymouth Reid* that in a solution of proteid no osmotic pressure can be traced to the proteid, and I find that when a solution of globulin is dialysed against water until equilibrium is reached, the dialysee and dialysate have exactly the same electric conductivity. These facts, added to others already stated, afford overwhelming evidence that in these solutions the chief portion of the proteid is, in effect, removed from solution by being gathered into masses of more than molecular dimensions which are separated from the rest of the solvent by a surface. The masses of probably hydrated proteid thus form an internal phase. In colloidal salts such as soaps, solutions of globulin in dilute acid or alkali, solutions of caseinogen or of acid or alkali albumen, in which the internal phase is bounded by double electric layers formed by ionic interaction with the external phase, the stability of the system with reference to forces such as gravity will depend, in part, upon the potential difference between the two faces round each particle. The electric double layers will contribute to stability, since any movement of a particle through the fluid will develop free electricity in quantity proportional to the potential difference between the layers.†

Salts in small amount diminish the stability of these colloidal systems and bring about concentration; and salts, as has been shown experimentally by Wiedeman, Perrin,‡ Burton,§ and others, diminish the potential difference in the condenser system round each particle.

The contribution which the electrification of the internal surfaces makes to mechanical stability is seen in the high viscosity of these solutions as

* 'Journ. of Physiology,' vol. 31, p. 438, 1904.

† Helmholtz, 'Wied. Ann.,' vol. 7, p. 337, 1879.

‡ 'Journ. de Chim. Physique,' vol. 2, p. 61, 1904; vol. 3, p. 50, 1905.

§ 'Phil. Mag.,' November, 1906.

compared with solutions of globulin by salts which resemble them in containing large molecules, but differ in the total absence of electric layers from these molecules.

Solutions of globulins in salts introduce us to an entirely different aspect of colloidal solution. Here, again, the proteid contributes nothing to the osmotic pressure,* and, therefore, must be withdrawn from true molecular solution; but as all ionic interaction is suppressed by the large excess of salt present, there are no double electric layers formed, and in their absence viscosity is low and stable equilibrium conditions are rapidly reached.

The origin of the mechanical stability of this system, the nature of the influence which maintains the even dispersion of the proteid throughout the fluid, is difficult to explain, since any factor to which we can appeal seems also to involve the exertion of a finite osmotic pressure by the proteid.

If the distribution of the energy be considered, we arrive at the following:—Let the state of thorough mixing of the components be established, and let a movement under the influence of any mechanical force take place, so that the proteid becomes more concentrated in some region. In this region, owing to the concentration of the colloidal salt, the degree of hydrolysis and of ionisation will be diminished, and the available energy thereby increased.

The equation of hydrolysis which Arrhenius† gives shows that hydrolysis and ionisation diminish faster than concentration increases; therefore, with unequal distribution of the proteid the gain in available energy ($E-\tau\phi$) will be greater in the regions of higher concentration than the loss in the regions of diminished concentration. The system as a whole, therefore, would gain in available energy, and the change from uniform distribution to unequal distribution could not take place unless work were done on the system.

* Waymouth Reid, *loc. cit.*

† 'Zeits. f. Physik. Chem.,' vol. 5, p. 16, 1890.